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Effects of Water Quality on Epithelial Morphology in the Gill of *Capoeta tinca* Living in Two Tributaries of Kizilirmak River, Turkey

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The fish gill is a multifunctional organ with complex external and internal organization. This organization is sufficiently flexible to allow fish to inhabit widely diverse and fluctuating environments (Laurent and Perry, 1991).Because they are directly in contact with the aquatic environment,the gills of fishes reflect tissue lesions which may be caused by waterborne pollutants.The complex and interconnected functional structural features of the gill epithelium, it makes an excellent model system to examine the effects of various dissolved substances on tissues (Evans, 1987).

This research was studied in Act Stream and Terme Stream which are tributaries of Kizilirmak River, Turkey between May 1995-June 1997. In this study, the morphological changes of the gill of *Capoeta tinca* an inhabitant of Aci Stream and Terme Stream were investigated as a function of water quality parameters.The catchment area of the Aci stream is covered mainly by lithologic units, containing highly soluble gypsum and salt rock, which make the water highly saline (2 ‰ to10.5 ‰). Aci Stream can not be used in irrigation because of its being very salty. Although it consists high rate of salinity, it has varieties of fishes which have economic importance. Terme Stream which has a large nutrition river basin, shows fresh water property and has different water quality and it gives a chance to compare with Aci Stream.

The chemicals, ammonia, nitrite, nitrate, ortho-phoshate and the high salinity were determined as parameters which possibly affect the gill morphology.

MATERIALS and METHODS

Due to the ecological differences between the selected sampling stations, fish specimens were caught by different methods. High salinity in Aci Stream did not allow electrofishing. Gill nets of 10-20 mesh size were use instead.The electrofishing method was successful in Terme stream. The fish specimens were measured for length proper and scales were collected and analysed for age determination in the field. Gills of 40 *Capoeta tinca* of Terme Stream and 11of Aci Stream were collected. Tissue specimens of the gills of each individual were fixed for twelve hours in Bouin solution and routinely embeded in paraffin wax for light microscopy. Sections were cut at 5 µm and stained with hemotoxylin and eosin(H&E).

Water temperature (T), specific electrical conductivity (EC), salinity (S), dissolved

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oxygen (DO), and pH were measured in the field. Water samples were collected seasonally and analyzed for the major ions and nutrients. All in-situ sampling preservation and analyses were made according to APHA standard methods (1989).

RESULTS and DISCUSSION

The chemical and physical characteristics of the streams are summarized in Table 1 and Table 2.

The amount of chlorine ions in the water can work as pollution indicator. The concentration of this ion in natural waters shouldn't exceed 20 mg/l. The results has indicated that, the chlorine concentration in Aci Stream was exceptionally higher than in Terme Stream (Table 2). High level of water chlorine in the water might indicates level of water pollution, although natural salt rock may contribute this high reading in this area.

The concentration of nitrate on surface waters is generally less than 1 mg/l (Anonymous,1981).The research results stated that the nitrate concentration in Aci Stream was much higher than the ones in Terme Stream.While the concentration is 1.67 mg/l in Terme Stream, it is 5.71 mg/l in Aci Stream (Table 2).

According to Ekmekçi (1989), Nisbet et Verneaux (1970) stated that the amonium concentration level higher than 1 mg/l indicates the conciderable level of water pollution. The concentration of amonium in Aci stream measured as 1.01 mg/l (Table 2), therefore the result may indicate amonium pollution in this area. The concentration of amonium in Terme Stream was 0.18 mg/l (Table 2).

The orto-phosphate is very important indicator of the water pollution.According to Ekmekçi (1989), Nisbet et Verneaux (1970) stated that the amount of orto-phosphate concentrations in natural waters shouldn't exceed 0.30 mg/l. Otherwise it would be the indicator of high level of water pollution.In Aci Stream concentration of orto-phosphate point it at scale of 0.28 mg/l, whereas the concentration of it in Terme Stream was 0.062 mg/l.

The concentration of salt in natural waters may also indicate water pollution (Anonymous,1981). Billard et Marie (1980) stated that *Capoeta tinca* and *Cyprinus carpio* resistance against high level of salt concentration in natural waters can not function over ‰9 of salt concentration. In summer season, while salt concentration was ‰10.8 in Aci Stream, it was ‰ 1.5 in Terme Stream.

In Aci Stream water quality might have been effected by several different inputs but mainly, intensive pesticide usage for agricultural purposes in surrounding area, the increase industrial activities and the increase in sewage inputs said to be the responsible for the water pollution in the area.In Terme Stream, the value of pollution indicators like ammonia, nitrite, nitrate, orthophosphate were very low so this expresses that in this station there is no pollution.

The histological structure of gill epithelium of control fishes is shown in Figure 1. Histological examination of gill tissues revealed that the filament epithelium of the *Capoeta tinca* of Terme and Aci Stream is different. Fewer chloride cells were

Table 1. Water quality parameters of Terme and Aci Stream measured in the field.

Station		
	Terme Stream	**Acı Stream**
Parameters	**Mean± SH (min-max)**	**Mean± SH (min-max)**
Temp (°C)	16.32±10.7 (n=8) (2 - 33)	18.84±11.73 (n=8) (2.8 -33)
DO (mg/l)	9.1±2.27 (n=8) (6.8-12,3)	8.98±1.59 (n=8) (7-11)
pH (25°C)	8.01±0.3 (n=8) (7.65-8.39)	7,93±0,34 (n=8) (7.44-8.42)
EC (µs/cm)	933.42±338.91 (n=8) (637-1570)	9573.57±5678.15 (n=8) (3028-18700)
S (‰)	0.71±0,52 (n=8) (0 - 1.5)	5.73±3.25 (n=8) (2-10.8)

Table 2. Mean values of the water analyses data obtained from Terme and Aci Stream (Ekmekçi, 1996)

Station		
	Terme Stream	**Acı Stream**
Parameters	**Mean± SH (min-max)**	**Mean± SH (min-max)**
SO_4(mg/l)	184.55±144.05 (n=8) (67.5-450.0)	1037.55±316.25 (n=8) (562.50-1400)
Cl (mg/l)	25.20±19.21 (n=8) (10.18-62.038)	3658.40±2689.48 (n=8) (563.65-7019.10)
Ca (mg/l)	71.65±21.90 (n=8) (50.7-112.22)	365.40±130.69 (n=8) (180.40-573.14)
Mg (mg/l)	48.93±16.05 (n=8) (29.18-76.60)	93.25±37.64 (n=8) (40.12-147.74)
HCO_3 (mg/l)	6.02±1.99 (n=8) (4.37-9.80)	4.28±0.86 (n=8) (3.35-5.35)
NH_3 (mg/l)	0.18±0.25 (n=8) (0-0.6)	1.01±0.50 (n=8) (0.48-1.91)
NO_2 (mg/l)	0.0082±0.02 (n=8) (0-0.055)	0.047±0.010 (n=8) (0-0.25)
NO_3 (mg/l)	1.67±1.23 (n=8) (0,66-3,32)	5.71±0.46 (n=8) (5.30-6.43)
o-PO_4 (mg/l)	0.062±0.052 (n=8) (0.005-0.13)	0.25±0.32 (n=8) (0.05-0.90)

observed in gill epithelium of *Capoeta tinca* of Terme Stream when compared to those of fish living in Aci Stream (Fig.2 and 3).

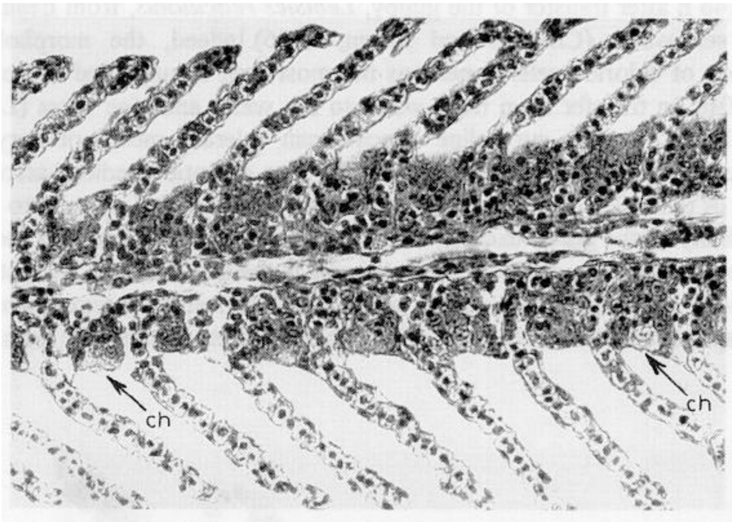

Figure 1. Histological structure of gill epithelium of control fishes caught from Terme Stream (X450 H&E).

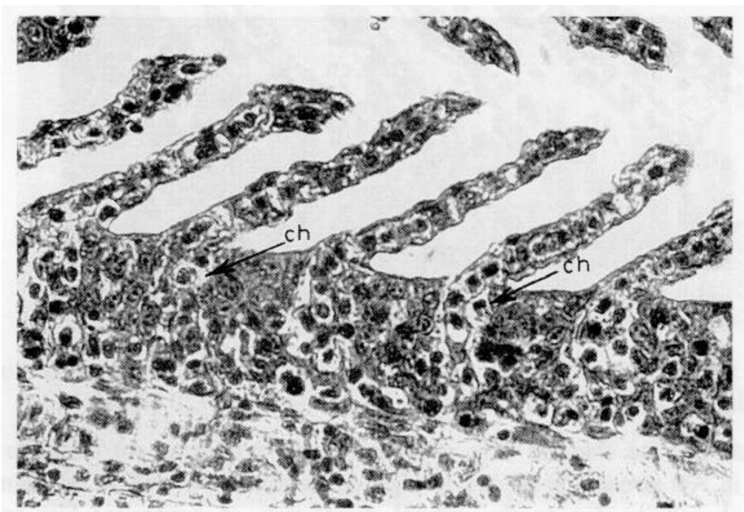

Figure 2. Histological structure of gill epithelium with a few chloride cells of *Capoeta tinca* caught from Terme Stream (X720 H&E).

The morphological adaptation of the fish gill structure to variable salinities has been the focus of intense resarch for many years, especially with respect to the role of the chloride cell. The chloride or mitochondria-rich cells of the gill play a prominent role in Teleostean fish's osmoregulation. Indeed, the permeability of thiscell type is modified according to the salinity of the external environment (Pisam, 1981). According to Eckert and Randall(1983), the number of chloride cells changed with changes in external salinity such that exposure to high salinities results in an increase in the number of chloride cells. The incorporation of 3H-thymidme into gill epithelial cells has revealed a doubling of filamental chloride cell production 48 h after transfer of the guppy, *Lebistes reticulatus,* from fresh water to 50 % sea water (Chretien and Pisam, 1986).Indeed, the morphological tranformation of chloride cells is perhaps the most well documented response of euryhaline fish on transfer from fresh water to sea water and vice versa (Laurent and Perry,l991). Certain euryhaline teleosts can tolerate media of very high salinity, greater than that of sea water it self The osmotic gradient across the integument of these fish is very high and the key to their survival appears to be the enhanced ability of the gill to excrete excess NaCl. It is well known that the most important osmoregulatory organ in these animals is the gill, which is characterized by a relatively slow absorbtion of Na and Cl in low salinity environmens, and relatively rapid secretion of Na and Cl in high salinity environments (Karnaky et al.,1976).

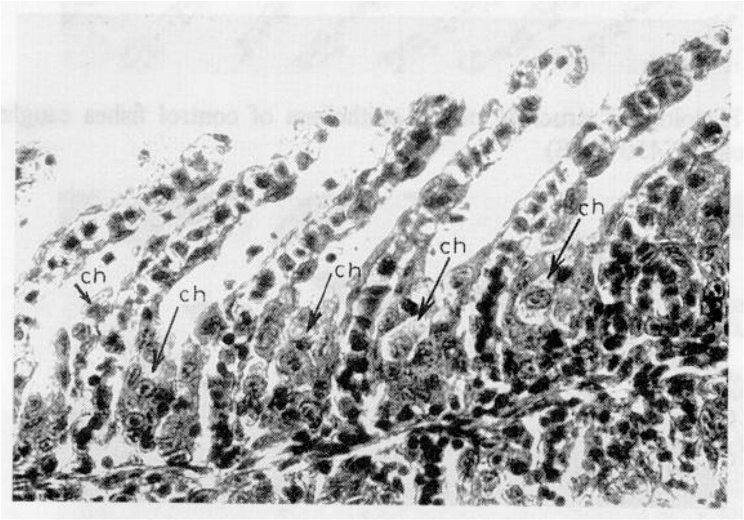

Figure 3. Histological structure of gill epithelium with increased number of chloride cells of *Capoeta tinca* caught from Aci Stream (X720 H&E).

The increase of the chloride cells observed in gill epithelium of *Capoeta tinca* which live in Aci stream is related to high concentration of salt in this sampling station High salinity due to the natural dissolution of evaporites like gypsum ($CaSO_4$) and rock salt (NaCl) appear to have induced many chloride cells in the gill filament epithelium perhaps as an adaption to extrem salt conditions for osmoregulation.Furthermore, chloride cell proliferation seems to reflect some role for chloride cells in toxicant extrusion or neutralization(Kamaky1980; Mallat et al. 1985).

Histopathologic lesions like formation of clavate lamellae(fig.3), lamellar fusion (fig.4), hyperplasia (fig.5) depending on pollution were observed in gill epithelium of *Capoeta tinca* which live in Aci Stream.

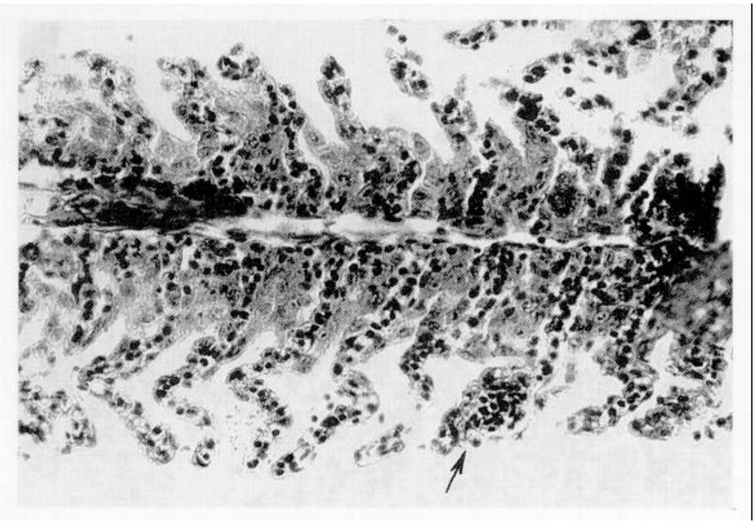

Figure 4. Histological structure of gill epithelium of *Capoeta tinca* taken from Aci stream showing clavate lamellae (X450 H&E).

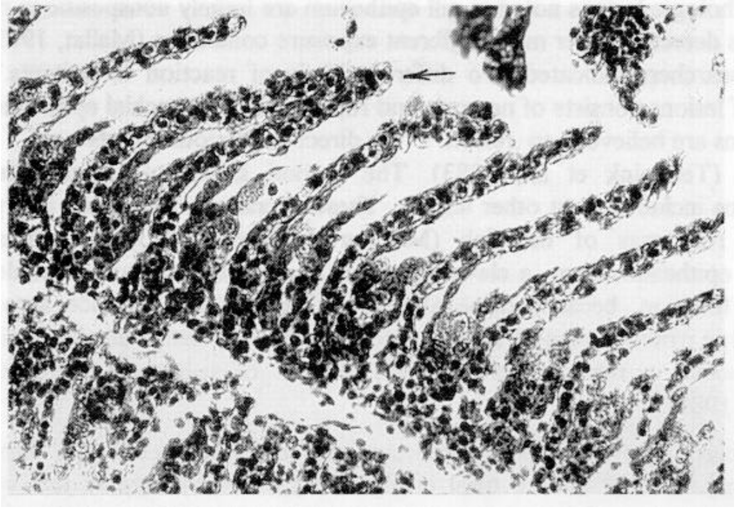

Figure 5. Histological structure of gill epithelium of *Caporta tinca* taken from Aci stream showing lamellar fusion (X750 H&E).

Intense hyperplasia formation has been observed in gills of *Lepomis macrochirus* exposing to concentration of 60 mg/l diazinon which is an organophosphorus insecticide (Dutta et al 1983). Richmonds and Dutta (1989) reported that there is a formation of lamellar fusion in gills of *Lepomis macrochirus* which is exposed to 0.05 mg/l malathion for 72 hours. Dutta (1993) observed the formation of clavate lamellae in gills of *Lepomis macrochirus* acting with the concentration of 75 mg/l diazinon for 24 hours.

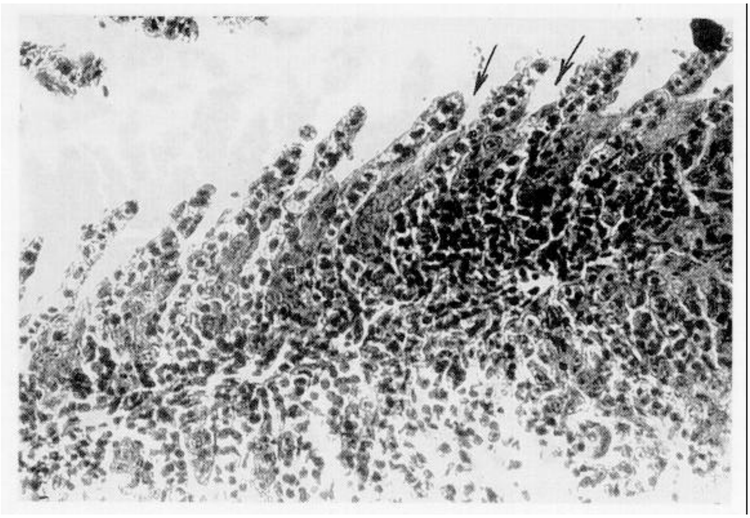

Figure 6. Histopathological structure of gill epithelium of *Capoeta tinca* showing hyperplasia (X450 H&E).

Histopathologic lesions noted in gill epithelium are largely nonspesific in nature, as each was detected under many different exposure conditions (Mallat, 1985). In the past, researchers indicated two different kinds of reaction to irritants.The first group of lesions consists of necrosis and rupture of the branchial epithelium. These alterations are believed to reflect 'the direct deleterious effects" of irritants on gills (Temmink et al., 1983). The second group of irritant-induced gill alterations includes most other lesions. These alterations have been interpreted as defense responses of the fish (Morgan and Tovell, 1973). Hyperplasia of the lamellar epithelium form a clavate lamellae as seen in this study could serve a defense function, because these alterations increase the distance across which waterborne irritants must diffise to reach the bloodstream. Lamellar fusion could be protective in that it diminishes the amount of vulnerable gill surface area (Mallat, 1985).

We conclude that, there is an important salt contamination in Aci Stream. This contamination leads to structural changes in gill morphology of fish which live there. Changes in gill morphology in response to a particular environment may be instrumental in conserving physiological functions. Moreover, these changes in gill morphology may contribute to the death of fish in contaminated waters.

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